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METHOD FOR TRANSPORTING ANIMAL CELLS
[Dobutsu Saibo No Yuso Hoho]

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[Claims]

[Claim 1] A method for transporting animal cells comprising: causing animal cells to form a cell mass, subsequently embedding said cell mass in a colloidal solution that assumes a sol state when heated and a gel state when cooled and whose conversion temperature between the sol state and gel state is in the range of from 4 to 39°C, and conducting the transportation or preservation of said cell mass at a temperature in the range in which this colloidal solution assumes a gel state.

[Claim 2] The method for transporting animal cells stated in Claim 1, wherein the colloidal solution has gelatin as its main component and the concentration of the gelatin is in the range of from 1 to 20 %.

[Claim 3] The method for transporting animal cells stated in Claim 1 or 2, wherein the colloidal solution contains a medium that sustains or proliferates the animal cells.

[Claim 4] The method for transporting animal cells stated in Claim 1 comprising: forming a collagen gel layer on the bottom of a container, cultivating animal cells on said collagen gel layer so as to form a cell mass, and subsequently embedding said cell mass, together with the collagen gel layer, in a colloidal solution.

[Claim 5] The method for transporting animal cells stated in Claim 4, wherein the collagen gel is a gel that is prepared by gelling

type 1 collagen from which telopeptide has been removed or type 1 collagen that has been treated with a reducing agent.

[Claim 6] The method for transporting animal cells stated in Claim 1, wherein the animal cells are primary-cultured hepatic cells or primary-cultured mammary cells.

[Detailed Description of the Invention]

[0001]

[Field of Industrial Application] The present invention pertains to a cell transportation and preservation method that is used in the fields of tissue culture, cell culture, and the like, mainly for transporting cells, and that, after the cell transportation, exhibits a high survival rate of animal cells and also makes it possible for the animal cells to sustain or express their function.

[0002]

[Prior Art] There are two types of cell transportation methods, one of which is to transport cells in a frozen state. The other is to cultivate cells in a culture-use flask, thus adhering the cells to the cultivation surface of the flask, and to seal tightly, with a stopper, the flask that is filled with a medium, under which condition the cells are transported.

[0003] The method of freezing to transport and preserve cells is widely applicable to both the adhesion system and suspension system, and, because established cell lines are less susceptible to damage caused by freezing compared with primary-cultured cells, the freezing

method is often used for transporting established cell lines. According to this method, cells are quickly frozen at 37 °C [sic], and restarting cultivation in an incubator can cause the cells to start multiplying.

[0004] With respect to primary-cultured cells, for transporting cells having strong proliferation potency, such as fibroblasts, epithelial cells, vascular endothelial cells, and the like, within a few days, the method of filling a flask with a medium is widely employed. Because the aforesaid primary-cultured cells are relatively strong against shaking, filling a flask with a medium so as to inhibit the movement of the medium during the transportation and to suppress the separation of the cells by agitation can make it possible to transport the cells without damaging them. The transportation is carried out at ordinary temperature, that is, at about 20 °C, and the cell growth is suspended during this period, but the cells start proliferating again once the filled medium is removed, a growth-use medium is added to the flask, and cultivation is started in an incubator at 37 °C.

[0005] Cells having strong proliferation potency can be transported and preserved by the aforesaid method, and they do not pose any problem as long as the objective is simply to transport cells. In recent years, however, it has been learned that some cells do not express the function of the cells proper when they are attached onto the cultivation surface of an incubator and cultivated in a single

layer, and the function of cells is expressed only when they form a cell mass and also learned that the structure that the cells construct is closer to the actual structure inside the body. Taking hepatic cells as an example, it has been reported that, by forming a cell mass, the cells sustain the ability to synthesize albumin for an extended period or sustain the drug-metabolizing function. It has also been reported that cancer cells, when they form a cell mass, have an intercellular structure similar to the structure of an actual cancer; as a result, their response to anticancer drugs and sensitivity to radioactive rays are closer to those of a cancer in the living body.

[0006] Meanwhile, there are various processes for forming a cell mass, but it takes a minimum of one week or so. Therefore, experiments that use cell masses require extra effort and time for an experiment preparatory step of preparing cell masses. If cells that are in the form of a cell mass could be transported, it would become possible to provide cell masses according to the needs of experimenters and thus to eliminate the need for the experimenters to prepare cell masses themselves.

[0007] A cell mass, however, is susceptible to damage caused by shaking during transportation and so forth, and its structure breaks down or the function of the cells cannot be sustained; for these reasons, it has not been possible to transport a cell mass. Primary-cultured hepatic cells are particularly susceptible to damage, and freezing or shaking kills them. Furthermore, even if the cells are

formed in a single layer in a flask and transported with less shaking, the hepatic cells can survive only for a week or so with a single-layer culture even under normal cultivation conditions. Accordingly, there is a need for a cultivation method that can transport and preserve the cells in mass form. In recent years, various types of cultivation kits that come with primary-cultured cells have been made available on the market, but, because of the aforesaid reasons, no kit for hepatic cells have yet been made available.

[0008]

[Problems that the Invention Intends to Solve] The present invention intends to solve the aforesaid problems that are encountered in forming and transporting a mass of animal cells, and its objective is to provide a method that is simple, without requiring special equipment; that does not damage cells during transportation; that, after the transportation, makes it possible to cultivate the cells while the function of the cells is maintained; and that is suitable for transporting animal cells and preserving them for a short period of time.

[0009]

[Means For Solving The Problems] The present inventors conducted intensive research to achieve this objective and, as a result, learned that animal cells, once they develop into a cell mass, are stable unless an impact or the like is applied to them; that a gel absorbs an impact and prevents a cell mass from breaking; that, by adjusting its

solute content, a colloidal solution of gelatin or the like can be made to maintain a gel state at a temperature of 35 °C or below and to become a sol quickly at 37 °C, which is the cultivation temperature of animal cells, thus facilitating its removal; that a colloidal solution of gelatin does not adversely affect cells; that the life of cells can be sustained by incorporating a medium in a colloidal solution and supplying nutrients to it; and so forth. Based on these findings, the present invention was achieved.

[0010] That is, the present invention is a method for transporting animal cells comprising: causing animal cells to form a cell mass, subsequently embedding said cell mass in a colloidal solution that assumes a sol state when heated and a gel state when cooled and whose conversion temperature between the sol state and gel state is in the range of from 4 to 39°C, and conducting the transportation or preservation of said cell mass at a temperature in the range in which this colloidal solution assumes a gel state.

[0011] The present invention is an animal-cell transporting method that is further characterized by the fact that the colloidal solution has gelatin as its main component and that the concentration of the gelatin is in the range of from 1 to 20 % (W/V); by the fact that the colloidal solution contains a medium that sustains or proliferates the animal cells; furthermore, by the fact that the method comprises: forming a collagen gel layer on the bottom of a container, cultivating animal cells on said collagen gel layer so as

to form a cell mass, and subsequently embedding said collagen gel and cell mass in a colloidal solution; and by the fact that the collagen gel is a collagen gel that is prepared by gelling type 1 collagen from which telopeptide has been removed or type 1 collagen that has been treated with a reducing agent.

[0012] The cell transporting method of the present invention is characterized by embedding in a colloidal solution a mass of animal cells formed inside an incubator and transporting or preserving the cell mass in a gel state. Among animal cells, the present invention exhibits excellent effects on primary-cultured hepatic cells and primary cultured mammary cells, and it is also applicable to pancreatic cells.

[0013] The colloidal solution may be any colloidal solution that assumes a gel state at an ordinary transporting temperature (room temperature) and dissolves readily at 37 °C, which is the common cell-cultivation temperature, to facilitate its removal and that is not toxic to cells, and an example of such a colloidal solution is a gelatin solution. Gelatin, in particular, does not change the structure and hardness of the collagen gel even when a cell mass is formed on a collagen gel. Furthermore, because gelatin is derived from collagen originally, its protein structure is similar to that of collagen, and, even if there is any residual gelatin, it does not affect the characteristics of the surface of the collagen gel; therefore, it is possible to restart cultivation directly from the

cell-mass state. Thus, it is ideal as the colloidal solution used in the present invention.

[0014] The following explains the method of the present invention, taking, as an example, the case of embedding primary-cultured hepatic cells in a gelatin colloidal solution. First, hepatic cells are collected from a rat or the like and formed into a cell suspension, and they are seeded and cultivated in an incubator that is coated with poly-HEMA (poly-hydroxyethyl methacrylate) or the like to impart cell-nonadhesive property to it or on a collagen gel. After a week or so of cultivation, hepatic cells form a cell mass. This cell mass is embedded in a colloidal solution of gelatin.

[0015] The following describes the preparation of the gelatin colloidal solution used for the embedding. The origin of the gelatin used here is not specifically limited, and the present invention can use products that are derived from porcine or bovine dermis or bones and that are commonly sold on the market and readily available. The suitable gelatin concentration of the prepared solution is from 1 to 20 % or thereabouts. If the concentration is thinner than this, the resulting gelatin jelly is not strong enough and could break during the transportation. With a concentration thicker than this, the viscosity of the gelatin solution increases, which leads to difficulty in dispensing the solution to embed a cell mass and in removing the gelatin solution to start cultivation.

[0016] In the gelatin solution, various kinds of mediums are added, and, since the cells are in a dormant state while they are being transported and preserved, it is sufficient to ensure minimal nutrients. The pH is adjusted to from 7 to 8, preferably to 7.4.

[0017] With the method for forming a cell mass on a collagen gel, the strength of the collagen gel proper needs to be increased. For this purpose, after a gelatin colloidal solution is dispensed in a container, it is recommended to carry out incubation at approximately 37 °C for several hours and replace the water in the collagen gel with the gelatin colloidal solution, after which the solution is solidified to form a gelatin gel layer.

[0018] On the other hand, in the case of not using a collagen gel layer for forming a cell mass, a gelatin colloidal solution may be dispensed in a solution that has a cell mass formed in it and then directly solidified to form a gel. An alternative way is to transfer the cell mass to a cell storage container and dispense a gelatin solution into it and solidify it.

[0019] After the cell mass is embedded in a gelatin gel, the container is sealed with a lid or the like for the purpose of preventing drying and contamination with bacteria and the like, under which condition a cultivation for the purpose of transportation and preservation is carried out. A gelatin colloidal solution assumes a gel state within a temperature range of from 0 to 30 °C or thereabouts and functions as a vibration- and impact-absorbing material; therefore,

vibration and impact that occur during transportation will not affect the cell mass, and the cells will not be damaged.

[0020] The following explains the method for restarting cultivation after the cell mass has been transported or preserved by the method of the present invention. If the method that forms a cell mass in an incubator to which a cell-nonadhesive property is imparted is employed, it is advisable not to perform cultivation again in the container used for the transportation and preservation. The reason for this is that, the gelatin is coated inside the container, and the cells adhere to it; consequently, the hepatic cells form a single layer, and the cell function deteriorates rapidly. When this method is employed, the following re-cultivation method should be used.

[0021] After the transportation and preservation, the used incubator as a whole is incubated at 37 °C. Since the gelatin gel layer dissolves quickly, the cell mass is taken out with a pipette or the like from the gelatin colloidal solution and transferred to a cell-nonadhesive incubator, such as a poly-HEMA coated incubator or the like, or onto a collagen gel, and a medium to be used is added to it to start a cultivation at 37 °C.

[0022] On the other hand, with respect to the case that employs the method that forms a cell mass on a collagen gel and embeds the whole collagen gel, including the cell mass, in a gelatin colloidal solution, incubation is carried out at 37 °C after the transportation or preservation to change the gelatin gel to a sol and to eliminate

the gelatin colloidal solution, and a culture medium is added and cultured for several hours at 37 °C, thereby replacing the gelatin colloidal solution in the collagen gel layer with the medium, after which an ordinary cultivation is carried out.

[0023] Because there is no cell damage during the transportation in either case, the re-cultivated hepatic cells have a high cell survival rate and maintain the form of a cell mass; thus, they can be cultivated for a long time while they maintain the cell function.

[0024] As a container for cultivating or for transporting and preserving animal cells, the present invention may use any incubator or storage container that is generally used for tissue culture or cell culture--for example, a petri dish, a plate having a plurality of wells, a flask, a cylindrical storage tube, and the like. The material of the container is not specifically limited as long as it is not toxic to cells.

[0025] With respect to the thickness of the gelatin gel layer into which a cell mass is embedded, there is no specific limitation for the method that does not use a collagen gel layer, provided that the layer is thick enough to completely embed a cell mass. For the method that embeds a cell mass together with a collagen gel layer, a thickness equal to or larger than that of the collagen gel layer makes it possible to reinforce the strength of the collagen gel layer and thereby to transport the cell mass.

[0026]

[Working Examples] The following explains the present invention in more concrete terms by presenting working examples.

Working Example 1

A 0.3 % acidic type 1 collagen solution, 10x PBS (a physiological phosphate buffered solution), and 0.01 N sodium hydroxide aqueous solution were mixed at a ratio of 8: 1: 1 aseptically under an ice-cooled condition, and 2 ml of the mixture was dispensed into a flask that was made from polystyrene resin and that had a 25 cm^2 cultivation area and heated at 37 °C, thereby forming a collagen gel layer. On this, the hepatic parenchymal cells that were collected from a rat (a Wistar rat, male, 5 weeks old) by a collagenase reflux [as transliterated] method were seeded in a quantity of 1 x 10^6 cells per petri dish, using an L-15 medium to which 10 % FBS (fetal bovine serum) had been added. Two hours after the seeding, a medium replacement was carried out. Thereafter, a medium replacement was carried out every two days to cultivate the cells for 7 days, thereby forming a cell mass of primary-cultured hepatic cells.

[0027] Meanwhile, a 10 % (W/V) concentration gelatin colloidal solution was formulated with pure water at 37°C, and an L-15 medium powder was added in a proportion of 14.8 g per 1 liter of the colloidal solution. The solution was subjected to sterile filtration and combined with sodium bicarbonate to adjust the pH to 7.4, thereby formulating a gelatin solution that contained an L-15 medium. Ten milliliters of this gelatin colloidal solution was dispensed into the

flask in which a cell mass had been formed, and a 10-hour incubation was carried out at 37 °C, thereby replacing the water in the collagen gel with the gelatin colloidal solution. At room temperature, the gelatin colloidal solution was gelled, thereby embedding the collagen gel and cell mass in the gelatin gel, and the flask thus prepared was subjected to a transportation test, after which it was subjected to a cultivation test.

[0028] Comparative Example 1

A sodium bicarbonate solution to which an L-15 medium powder was added in a proportion of 14.8 g/l and that was then subjected to sterile filtration was adjusted to have a pH of 7.4, and this solution was packed in a flask in which was formed a cell mass in the same manner as in Working Example 1, and the flask thus prepared was subjected to a transportation test, after which it was subjected to a cultivation test.

[0029] Comparative Example 2

A solution that was prepared by aseptically diluting a 0.3 % acidic type 1 collagen solution with RBS 10 times was dispensed into a flask that was made of polystyrene resin and that had a 25 cm^2 cultivation area in a quantity of 5 ml and heated at 37 °C, thereby forming a collagen coating layer. On this, the liver parenchymal cells collected in Working Example 1 were seeded and cultivated under the same conditions. The obtained cultured cells formed a single layer. This flask was filled with a sodium bicarbonate solution that was

prepared in the same manner as in Comparative Example 1, and the flask was sealed and subjected to a transportation test, after which it was subjected to a cultivation test.

[0030] Comparative Example 3

Culture of a cell mass that was formed in the same manner as in Working Example 1 was continued as is at 37 °C, using the same medium (an L-15 medium to which 10 % FBS was added).

[0031] The flasks prepared in Working Example 1, Comparative Example 1, and Comparative Example 2 were transported 1,300 kilometers by a truck for 3 days, after which the cell conditions were investigated. With respect to Working Example 1, after the transportation, the flask was heated to 37 °C to dissolve the jelly layer and eliminate the gelatin solution. Thereafter, a 10 % FBS-added L-15 medium to which insulin and glucagon had been added was put in the flask, and cultivation was carried out at 37 °C. With respect to Comparative Example 1 and Comparative Example 2, after the transportation, the aforesaid medium was added to the flask, and cultivation was carried out at 37 °C. Incidentally, with respect to Comparative Example 3, the flask was not transported, and the cultivation at 37 °C was continued, using the aforesaid medium.

[0032] For each specimen, determination of the number of viable cells, a cell function test, and so forth were conducted according to the following procedures.

(1) Determination of the number of viable cells and observation of the cell form

An ordinary cultivation was started after the transportation, and, 12 hours later, the number of viable cells was found and expressed as a percentage, assuming the number of viable cells in Comparative Example 3 to be 100.

(2) Cell function test

The albumin content in the medium was measured at 3 days, 7 days, and 15 days after restarting the ordinary cultivation (that is, 13 days, 17 days, and 25 days after the collection of the cells) and expressed as a percentage, assuming the albumin content in Comparative Example to be 100.

[0033] The results of the observation and measurement of each specimen are shown in Table 1, and it is evident that the present invention's method for transporting primary-cultured hepatic cells was capable of transporting the hepatic cells without damaging them and that the function of the hepatic cells was maintained without any change even after the transportation.

[0034]

[Table 1]

TABLE 1: TRANSPORTATION TEST RESULTS WITH RAT HEPATIC CELLS

	Work. Ex. 1	Comp. Ex. 1	Comp. Ex. 2	Comp. Ex. 3
Comparison of No. of viable cells	98	65	24	100
Cell Form	The cell mass was maintained.	The cell mass was partially maintained, but, as a whole, the breaking of the mass was observed	A single layer was partially maintained. Separation was observed.	A cell mass was formed
Comparison of albumin content in the medium				
13 days after collection	80	12	0	100
17 days after collection	98	8	0	100
25 days after collection	103	5	0	100

[0035]

[Effects of the Invention] With the use of the present invention's method for transporting animal cells, the transportation and short-term preservation of animal cells in a cell-mass form at ordinary temperature, which has been considered to be impossible, becomes possible, without using any special equipment and without losing the cell function. Furthermore, this method makes it easier to restart an ordinary cultivation. Thus, it is ideal as a transportation method that is intended for transporting and preserving animal cells in mass form.